THE CHARACTER OF THE FLAGELLA.

DR. VERANUS A. MOORE.

The Character of the Flagella on the Bacillus Cholera Suis (Salmon and Smith), Bacillus Coli Communis (Escherich), and Bacillus Typhi Abdominalis (Eberth), Wilder Quarter-Century Book. 1893.

The prediction of Ehrenberg, that motile bacteria are possessed of flagella upon which their power of locomotion depends, was partially fulfilled, as early as 1875, by Dallenger and Drysdale, who saw these hair-like appendages lashing on a moving, living bacterium ; but owing to the difficulties in the way of demonstrating these exceedingly minute filaments, our knowledge as to the character of the flagella is very meagre and somewhat contradictory.

Cohn (1872), and Koch (1877), appear to have succeeded in staining the flagella on a few of the larger saprophytes, but not until Loeffler perfected his method, the essential feature of which is the use of a mordant, was any knowledge obtained as to the flagella of the small, especially pathogenic bacteria.

Soon after the publication (1889) of Loeffler's method, by which flagella on a considerable number of motile bacteria was demonstrated, Messia proposed a new classification of bacteria based upon the number and arrangement of their flagella. It is as follows :

I. GYMNOBACTERIA (non-motile).

II. TRICHOBACTERIA (motile).

1. *Monotricha*, having one flagellum at one pole of the bacillus.
2. *Lophotricha*, having bunch or tuft of flagella at one pole.
3. *Amphitricha*, having a flagellum at each pole.
4. *Peritricha*, having rows of flagella.

This classification is based on morphological characters only, and recognizes genera, but not species. But bacteriology recognizes functional properties in the formation of species and varieties, and there are bacteria which possess marked differences in their biological manifestations which resemble each other so closely in their structure that, with our present knowledge, their differentiation by purely morphological characters, is practically impossible. Luksch found a specific difference in the number of flagella possessed by the colon and typhoid bacilli, two well recognized species of the same genus, and made use of this information in differentiating between these closely allied species. These and the hog-cholera bacillus form a group which resemble each other very closely morphologically, but are readily differentiated by their biological and etiological properties, and consequently afford a rigid test for the differential value of the flagella. They were, therefore, chosen by the author as the basis of the work herein presented, and which was directed along the following lines :

1. To complete as far as possible our knowledge of the morphology of each of these organisms.

2. To determine whether or not there is a difference in the number or character of the flagella of the modified forms of these bacteria corresponding with the variations that are found to exist in their physiological and etiological manifestations.

3. To determine the significance of the flagella in classifying motile bacteria, as illustrated by a comparative study of these filaments on three typical species, representing morphologically three closely allied groups, and biologically three distinct groups, of bacteria.

Two cultures, from different sources, of each of the three species were used in making the cover-glass preparations, and accurate counts, measurements and observations were made of two hundred individual germs from each culture, selected from somewhat ideal fields so as to avoid extreme conditions and obtain as accurate results as possible.

The following tables give the results in a way which admits of easy comparison :

A COMPARISON OF THE NUMBER OF FLAGELLA ON THE INDIVIDUAL GERMS.

BACILLUS.	Culture	The Number of Flagella.									Total number of bacteria.	Average number of flagella on each germ
		0	1	2	3	4	5	6	7	8	9	
Cholera suis	(1)	12	23	30	47	39	22	12	8	5	2	200	3.3
" "	(2)	10	33	33	45	38	19	6	8	6	2	200	3.1
Coli communis	(1)	9	33	58	44	34	15	4	3			200	2.6
" "	(2)	11	83	55	29	13	6	3	(?)			200	1.8
Typhi abdominalis	(1)	9	23	39	45	27	15	23	11	3	3	200	3.5
" "	(2)	17	43	42	45	24	18	5	3	3	3	200	2.6

A COMPARISON OF THE LENGTH, DIAMETER AND CHARACTER OF THE FLAGELLA.

BACILLUS.	Culture	Length of longest flagellum.	Lgth. of 70 per ct or more of the flagella.	Usual diameter of flagella.	Appearance of flagella.
Cholerae suis . .	(1)	18 μ	7–12 μ	0.1–0.2 μ	Usually extended, wavy, few terminal rings.
" "	(2)	11 μ	6–8 μ	0.1–0.2 μ	" " "
Coli communis . .	(1)	12 μ	5–7 μ	0.1–0.2 μ	" " "
" " . .	(2)	15 μ	5–9 μ	0.1–0.2 μ	" " "
Typhi-abdominalis	(1)	11 μ	3–6 μ	0.1–0.2 μ	Many incurved, wavy, large number of terminal rings.
" "	(2)	13 μ	3–7 μ	0.1–0.2 μ	" " "

From the foregoing, and as the result of a careful comparative study of the flagella of three species of bacteria the author reaches the following conclusions :

1. These three species of bacteria belong to the *Peritricha* (Messea).

2. There are apparently slight differences in their flagella, but the differences are not marked enough to be of differential value.

This is evidenced by the fact that the flagella in different preparations from the same species exhibit quite as marked variations.

3. There is no difference in the flagella of modified forms of the same species to correspond with the difference in their physiological and etiological manifestations.

4. Until further facts are determined, the character of the flagella will not furnish a means for specific differentiation. The species and varieties must be determined by their physiological and pathogenic properties while the genera may be fixed by the character of the flagella.

5. The proposition that the *Bacillus typhi abdominalis* is a modified form of *Bacillus coli communis* cannot be justly refuted on their morphological characters. (But the author doubts the truth of this claim.) The similarity in the structure (as it is now understood), of these bacteria increases the importance, from a differential standpoint, of the differences found to exist in their biological and etiological manifestations.

For staining the flagella the author has used Loeffler's method with modifications.

As a mordant he used:

A 20 % solution of tannic acid,	10 c.c.
A cold saturated solution of iron sulfate,	5 c.c.
A saturated alcoholic solution of fuchsin,	1 c.c.

As a staining fluid; Ziehl's Carbol Fuchsin.

Fuchsin,	1 gm.
Absolute alcohol,	10 c.c.
A 5 % solution of carbolic acid,	100 c.c.

The fuchsin is dissolved in the alcohol, after which the acid is added.

The cover-glass preparations:—The cover-glasses must be absolutely clean. A large drop of *warm* water is placed upon each by means of a sterile pipette. The point of a sterile platinum wire is gently touched to the culture (on a jar or gelatin), and immersed in the water near the center of the cover-glass. A sufficient number of bacteria will adhere to the wire to make from 6 to 10 prearations. The tray containing them is placed in an incubator at 36° C., until the water has evaporated. Many of the bacteria on account of their power of locomotion and the cur-

rents produced in the liquid during evaporation will be isolated from the clumps introduced on the wire, and will be evenly distributed over a large portion of the cover-glass. After the preparations have dried, they are fixed by passing them twice through the flame of a Bunsen burner, or, better, by heating for from five to ten minutes in the hot air oven at 120° to 140° C. The c vers are now immersed in 3 or 4 c.c. of the mordant in a large test tube. This is heated until steam begins to come off. It is then removed from the flame and gently agitated for five or ten minutes when the covers are washed in water. If there is a grayish film on the cover it can generally be removed by rinsing in alcohol and then again in water. The staining fluid is now applied in the same way as the mordant and allowed to act for from one to three minutes.

The mordant should be fresh and always filtered before use. The success will depend much upon the strict adherence to details. The mordant and stain may be mixed and used in one solution, but must act for a much longer time. Fuchsin gives better results than any of the other aniline dyes. No other mordant has been found that will take the place of tannic acid. This should, when possible be procured chemically pure. Slightly better esults are obtained with the typhoid and colon bacteria, when a few drops of a ten per cent. solution of sulphuric or acetic acid are added. The staining process increases to a slight extent the size of the body of the germs, either by causing the cellular substance to swell or by staining a capsular substance surrounding the body, which is not brought by the ordinary staining processes. The flagella are hair-like appendages or filaments which radiate from the bacteria and ore given off from the cell wall of the germs of which they appear to be continuations or projections. Bacteria will always be found in the finished preparations having no filaments, and there will also be loose filaments and clumps of filaments ; but fields can also be selected in which all of the bacteria are provided with motile appendages in which there are no loose filaments. The filaments presumably become detached in the manipulation of the germs, and probably to some extent during life from becoming entangled with each other.

The longest flagellum observed was 18 μ, or about nine times

the length of the body of the bacillus. They vary greatly in length. In diameter they range from 0.4 μ to 0.2 μ, or about one-third to one-sixth the diameter of the body of the germ, the latter being the more common size. The author was never able to see the flagella in unstained preparations.

DESCRIPTION OF PLATE.

The figures in the plate are to illustrate the flagella on these three species of bacteria as they appeared in stained cover-glass preparations. The drawings were made by the aid of a Zeiss apochromatic objective, 2 mm., 1.30 n. a. and the measurements were made with the compensating micrometer ocular No. 6. Each germ and its flagella were carefully measured and in the drawings each micromillimetre is represented by a millimeter, thus giving a magnification of a thousand diameters. The curves in the flagella were carefully counted and reproduced as accurately as it was possible by free-hand drawing. The position of the flagella were also carefully determined. In the preparation of the plate care has been taken to avoid extremes. Individual bacteria have been selected from difierent fields to represent the various number, lengths and position of the filaments on the body of the germs as they appeared in the preparations. A few free, or detached flagella are also indicated. The drawing of each germ is practically equivalent to a photograph. It is possible to find all of the structures represented in a few fields of the microscope in a well executed preparation. The germ in the center of each figure represents the maximum number of flagella on a single individual. In the left lower corner of each is a drawing of a clump of bacteria with their flagella. There are a few drawings of bacteria (*a*) with only their periphery and flagella stained.

Fig. 1. *Bacillus choleræ suis.* Drawings made from preparations of the culture of hog cholera bacteria obtained in the State of Illinois. (*b*) A bunch or strand of flagella.

Fig. 2. *Bacillus coli communis.* Drawings made from preparations from the culture obtained from the human intestine.

Fig. 3. *Bacillus typhi abdominalis.* Drawings made from preparations of the typhoid bacillus which was obtained from the Johns Hopkins Hospital. The upper right hand corner, enclosed in dotted lines, represents all of the bacteria and flagella from a single microscopic field.

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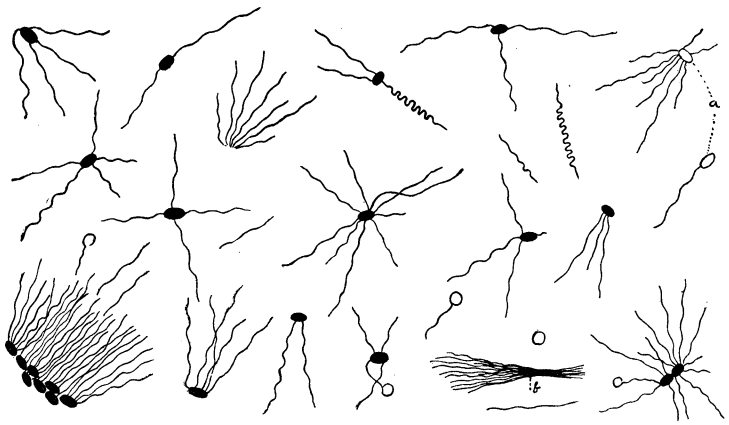

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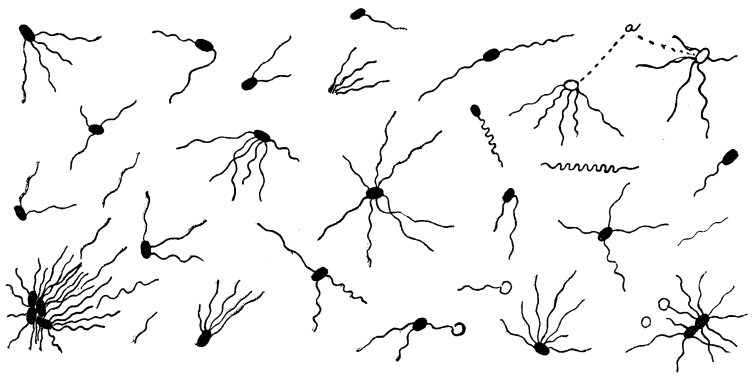

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